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SWEAT GLAND FUNCTION IN ISOLATED PERFUSED SKIN

BY K. G. JOHNSON*

From the A.R.C. Institute of Animal Physiology, Babraham, Cambridge, CB2 4AT

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SUMMARY

1. A technique for perfusion of skin has been used to investigate a possible neurochemical basis for the different patterns of sweating in domestic animals. Evaporative water loss was measured from excised trunk skin, ears or tails perfused with a nutrient Krebs solution, to which drugs were added as required. Perfused skin was observed to sweat in response to administration of sudorific drugs, and some features of the patterns of sweating were similar to those which could be induced by heating or by drugs in conscious animals.

2. In sheep and goat skin, injections of adrenaline, and to a lesser extent of noradrenaline, elicited brief sweat discharges but these were not sustained when the drugs were infused during 10–20 min. Injections of isoprenaline, carbachol, 5-HT, bradykinin, oxytocin and histamine were all ineffective.

3. Injections of adrenaline into cattle skin evoked longer-lasting sweat discharges, and infusions of adrenaline elicited continuous discharges. Injections of noradrenaline and sometimes of bradykinin caused only brief sweat discharges; other drugs were ineffective.

4. In horse and donkey skin, injections or infusions of noradrenaline, oxytocin and bradykinin elicited brief discharges of sweat. Infusions of isoprenaline caused a continuous and profuse outflow of sweat. Infusions of adrenaline also caused a continuous discharge which was usually biphasic in its onset. Other drugs were ineffective.

5. Assuming that the brief sweat discharges are due to myoepithelial contractions and the continuous discharges to sustained increases in secretion, equine sweat glands seem to have an α-adrenergically controlled myoepithelium and a β-adrenergically controlled secretory mechanism. Sheep and goats may have a similar α-adrenergic control of the sweat gland

* Present address: Department of Veterinary Biology, Murdoch University, Murdoch, W.A. 6153, Australia.

myoepithelium but only a feeble sweat secretory mechanism. In cattle, an α-adrenergic mechanism appears to control sweat secretion, but the control of the myoepithelium is uncertain.

INTRODUCTION

Sweat glands of domestic animals discharge in patterns characteristic of the species. In sheep and goats, exposure to heat evokes intermittent pulsatile ejections of sweat which occur simultaneously over the body surface (Bligh, 1961); no continuous sweating occurs except when consecutive discharges overlap (Robertshaw, 1968; Johnson, 1971). In cattle, regular discharges similar to those seen in sheep occur, but they are superimposed on a continuous output of secretion (McLean, 1963). In the horse and donkey, the output of sweat is also continuous but such fluctuations as occur are less obviously synchronous and are of variable form (Allen & Bligh, 1969; Robertshaw & Taylor, 1969).

The simplest explanation for the different patterns of sweat discharge is that sweat output depends on two or more mechanisms which are not equally effective in different species. Bligh (1967) suggested that the sweat discharge on to the skin could result from a simple overflow due to an increased rate of secretion, or to an intermittent squeezing of the gland lumen by the myoepithelium which invests it. Muto (1916) had suggested that the sweat glands of the horse, ox and sheep are innervated and controlled by both sympathetic and parasympathetic nerves, and more recent histochemical evidence of the presence of enzymes from both adrenergic and cholinergic nervous systems in the sweat glands of cattle (Jenkinson, Sengupta & Blackburn, 1966) and horses (Jenkinson & Blackburn, 1968; Bell & Montagna, 1972) also indicates the possible involvement of two neural influences on sweat gland activity.

When drugs are given intravenously to study the effects on sweat gland activity, their actions are not necessarily directly on the glands or the neuroeffector junctions but could be of central neural or humoral origin. Foster, Ginsburg & Weiner (1970) have used intradermal injections beneath a sweat-detecting capsule to study the local effects of drugs on human sweat glands, but this technique is unsuitable for animal work unless general anaesthesia is used, and then additional difficulties may be introduced. An alternative approach is to perfuse the blood vessels of an isolated piece of skin and to introduce drugs into the perfusate. This technique was shown by Johnson & Linzell (1972) to be feasible, and has been used in the present study of the direct effects of putative transmitter substances and other drugs on the sweat glands of domestic animals.

METHODS

Two series of experiments with the same objective but using slightly different techniques were done. In the first, partially described by Johnson & Linzell (1972), the ears and mammary or inguinal skin of 11 sheep and nine goats of various breeds, and the trunk skin and ears of four Jersey cows were collected from animals which had been heparinized and then anaesthetized with sodium pentobarbitone. Tissues were taken from animals sometimes before and sometimes after exsanguination. Skin perfusions were made with constant pressure from a reservoir of Krebs solution fixed 1·36 m above the tissue. Flow of perfusate was measured with a drop-counter and the drugs were added to the perfusate at the point where it entered the tissue. The disadvantage of this system was that vasoconstrictive drugs caused a virtual cessation of the perfusion.

In a second series of experiments, perfusion was by constant-volume inflow using a peristaltic pump; vasomotor changes were then monitored by changes in perfusion pressure. In this series, the flank skin, ears or tail of nine sheep, five goats and five cattle and the inner or outer thigh or shoulder skin of three horses and one donkey were perfused. The animals were of assorted breeds. Most animals were killed with an overdose of anaesthetic agent, but two sheep were stunned electrically and then exsanguinated and the tails of two oxen were collected at abattoirs where the animals were killed with a captive bolt and then pithed and exsanguinated. Sweat glands in the skin from stunned sheep were almost insensitive except to high doses of drugs, perhaps because the glands discharged immediately after stunning; this was subsequently demonstrated with a starch-iodine mixture on the skin (Wada, 1950). Sweat glands in the skin from stunned cattle were more responsible than those of stunned sheep, possibly because the sweat glands of cattle have a greater capacity for secretion.

Perfusion of skin samples was commenced as soon as possible, usually 1–3 hr after collection. In some experiments, tissues were found to be still responsive to drugs 12 hr after collection, but after 24 hr storage at 4° C the sensitivity was greatly reduced.

Arteries suitable for cannulation with blunted hypodermic needles (0·80–1·65 mm o.d.) were easy to find in the trunk skin of the horses and the donkey; in cattle, sheep and goats, arteries in trunk skin suitable for cannulation even with fine needles (0·46 mm o.d.) were rare. Therefore only sixteen of the twenty-seven perfusions of ruminant skin in the first series of experiments and four of the thirty perfusions of ruminant skin in the second series were made through small arteries of the trunk, mammary or inguinal skin; the remainder were made through the large central arteries of the ears or tail.

The arrangement used for perfusion in the first series of experiments is described by Johnson & Linzell (1972). For the second series of experiments the tissue was arranged in the system illustrated in Fig. 1. Sheets of skin from the trunk were mounted, outer surface down, over a rigid brass ring (20 or 30 cm diam.) and held with a flexible metal ring clamped with a tangentially mounted bolt (Fig. 1*a*). To prevent drying of the tissue, a thin polyethylene sheet was drawn over the subcutaneous surface and held with a large elastic band. Ears or tails were enclosed almost entirely in a cylindrical container sealed at the lower end with an annular sheet-rubber diaphragm (Fig. 1*b*). In ear perfusions, the external auditory meatus was blocked with a rubber bung.

Skin temperature (T_{sk}) was maintained between 24 and 35° C with two voltage-controlled lamps on opposite sides of the tissue, and measured with thermocouples placed on the skin surface and/or subcutaneously. Water evaporated from the skin

surface was collected either with a capsule (64 mm i.d.) attached with adhesive to a clipped trunk skin surface in the region being perfused (Fig. 1*a*) or from the entire ear or tail (Fig. 1*b*). Evaporative water loss (EWL) was estimated in both systems from the difference between the wet bulb temperatures of room air and of air drawn through the capsule or cylinder (Allen & Bligh, 1969). The air flow through the capsule was maintained at 1 l/min, but over the larger areas of skin on the ears or tails, flow was increased as required to 2 or 3 l./min. The sweat measuring systems were calibrated by infusing water at known rates into the capsule or container.

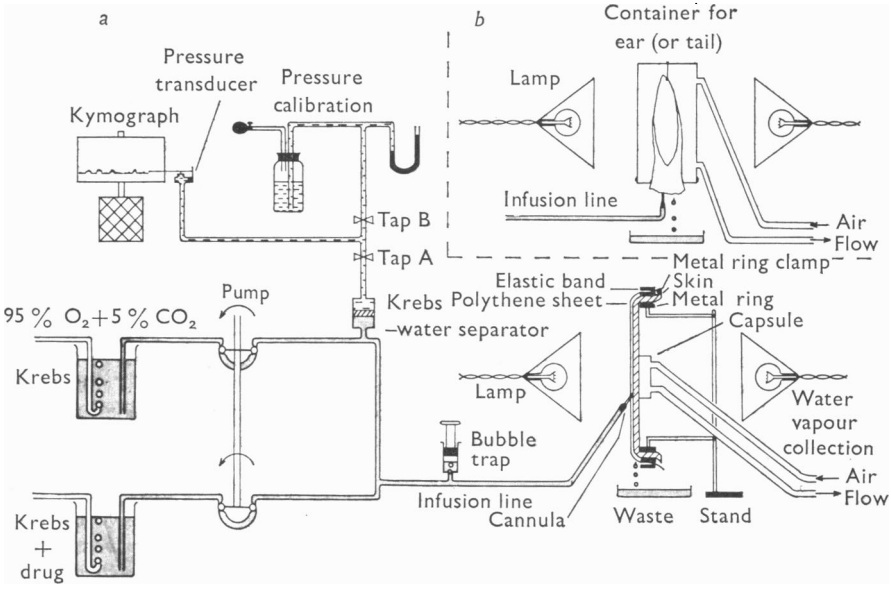

Fig. 1. Schematic diagram of the perfusion system (*a*) for trunk skin and (*b*) for an ear or tail. Tap A is closed during calibration of the pressure recording system; Tap B is closed during perfusion.

The tissues were perfused with a modified Krebs solution containing 6·73 g NaCl, 0·263 g KCl, 0·300 g $MgSO_4.7H_2O$, 0·163 g KH_2PO_4, 2·1 g $NaHCO_3$, 0·5 g $CaCl_2.2H_2O$, 0·6 g glucose and 0·05 ml. glacial acetic acid per litre which was passed through a 0·3 μm Millipore filter then gassed for 1–2 hr with 95 % O_2–5 % CO_2 and finally adjusted to pH 7·4 with NaOH; gassing was continued during perfusion.

The adjustable peristaltic pump (Watson–Marlow MHRE/2, Falmouth, England, or Desaga Peristaltic Pump, Heidelberg, Germany) pumped the perfusate from two reservoirs. One reservoir always contained unmodified Krebs solution; the other did also except when a drug was to be administered when it was replaced by a Krebs solution containing the drug. This was done either for a short time (up to 1 min) to study the effect of a single injection of the drug, or continuously for 10–30 min to study the effect of sustained infusion of the drug. The flows of perfusate through the two perfusion lines were merged, passed through a bubble trap and connected to the arterial cannula. From the line which contained Krebs solution only, a side-arm led to a 5 ml. sealed glass syringe containing a piston which served to separate the perfusate on one side from distilled water used in a pressure recording system on the

other. Changes in pressure were transmitted to a mechanical transducer which responded to small changes in volume (100 mmHg per 1 ml. displacement) and controlled a pen writing continuously on a kymograph. A U-tube of mercury, a pressure bottle and an inflation bulb were attached to the circuit for pressure calibration.

The drugs used were (−) adrenaline bitartrate (Sigma), (−) noradrenaline bitartrate (Sigma), isoprenaline sulphate B.P. (Evans), 5-hydroxytryptamine creatinine sulphate (Sigma), carbachol (B.D.H.), histamine acid phosphate (B.D.H.), synthetic bradykinin (Sandoz) and oxytoxin (Pitocin, Parke Davis). The drugs were dissolved in Krebs solution containing 50 mg/l. (−) ascorbic acid as an antoxidant. The doses reported refer to the salt. For each species, each drug was introduced at least three times into preparations of perfused skin taken from two or more animals. Occasional injections of atropine sulphate and pempidine tartrate (Fig. 5*a*) were also given but their effects were not studied systematically.

RESULTS

Perfusion pressure, perfusion flow, T_{sk} and EWL

In the first series of experiments at constant pressure (100 mmHg), perfusion flow varied between zero and 0·5 ml./min in trunk skin and 40 ml./min in mammary skin. In the second series at constant-volume inflow, the pressure at the arterial cannula was adjusted to 100 mmHg during administrations of unmodified Krebs solution; following administration of vasoconstrictive substances perfusion pressure rose, sometimes to more than 200 mmHg. In sheep, goat and cattle skins, the flow of perfusate ranged from 1–2ml./min through fine trunk skin vessels to 6·4–20·0 ml./min through the ear or tail. In the horse and donkey skin, perfusion rates through trunk skin vessels were 2·0–8·6 ml./min.

T_{sk} remained relatively constant unless vasoconstriction was induced by the administration of a drug. Then a slowing of perfusion led usually to a rise in T_{sk} which increased EWL independently of any sweat discharge. To allow for this effect, EWL was measured on three occasions for each species (except the donkey) while T_{sk} was varied by altering the heating intensity of the lamps, and the relation between EWL and T_{sk} was determined. The mean changes in EWL which occurred as T_{sk} changed are recorded in Table 1, and only changes in EWL greater than these have been considered to be due to sweating. If the perfusion pressure was decreased to 50 mmHg or increased to 200 mmHg, increases or decreases in T_{sk} occurred and these led to changes in EWL. However, when allowance was made for the concomitant changes in T_{sk}, no further change in EWL could be attributed directly to the alteration in perfusion pressure, therefore no corrections to EWL were necessary when changes in perfusion pressure were induced by vasoactive drugs.

The basal rates of EWL from skin perfused by Krebs solution and unstimulated pharmacologically are listed in Table 1, as are the rates of

TABLE 1. Basal rates of EWL, changes in EWL with T_{sk}, and sweating responses of isolated perfused skin to single injections or continuous infusions of various drugs. Sweating responses are graded as: ††† = profuse, †† = moderate, † = slight, or * = absent. Figures for EWL and $\Delta EWL/\Delta T_{sk}$ are mean ± S.E. of mean; drug doses are ranges. Figures in brackets are numbers of observations

	Sheep	Goat	Cattle	Horse	Donkey
Basal EWL ($mg.m^{-2}.s^{-1}$)	4·8 ± 0·6 (14)	8·2 ± 1·6 (16)	7·6 ± 0·9 (13)	11·1 ± 2·4 (5)	4·4 (1)
$\Delta EWL/\Delta T_{sk}$ ($mg.m^{-2}.s^{-1}$ per ° C)	0·81 (4)	0·47 (4)	0·33 (3)	0·68 (2)	—
Adrenaline bitartrate					
Single (μg)	††0·50–50 (26)	††0·05–21·6 (23)	††1–50 (20)	*0·01 (1) †††1–6 (2)	††1·5 (1) †††3 (3)
Continuous (μg/min)	††2–13·5 (6)	††1·6–13·5 (4)	††4·3–65 (5)	*0·01 (1) †††0·1–0·43 (3)	—
Noradrenaline bitartrate					
Single (μg)	*5–10 (2) †0·1–5 (3)	*5–68 (3) †68 (2)	*13 (1) †1·5–10 (5)	*0·1 (1) †10–36 (3)	†3 (1)
Continuous (μg/min)	*13·5–22 (2) †2·5–21 (3)	†1·6–5 (4)	*4·3–5 (2) †65–72 (2)	*0·1–1 (2) †0·43–4·3 (2)	—
Isoprenaline sulphate					
Single (μg)	*5–100 (3)	*1·8–10 (3)	*1–2 (3)	†††0·1–1·0 (3)	†††1·5 (1)
Continuous (μg/min)	*10–43 (3)	*1·35–20 (4)	*3·8–13 (3)	†††0·1–10 (4)	—
Carbachol					
Single (μg)	*5–100 (2)	*10–68 (3)	*0·2–20 (6) ?2·0 (1)	*1–10 (3)	—
Continuous (μg/min)	*10 (2)	*1·35–20 (3)	*43–80 (3)	*4·3–10 (2) †17·5–175 (2)	—
5-HT creatinine sulphate					
Single (μg)	*5–20 (3)	*2·2–32 (4)	*1–43 (3)	*2–10 (3)	—
Oxytocin					
Single (units)	*0·6–1 (3) ?1·0 (1)	*0·22–1·64 (4)	*1–2 (3)	†1–1·75 (3)	—
Bradykinin	*1·25–10 (4)	*2·5–3·2 (2)	*2–5 (2)	†1–2 (3)	?1·5 (1)
Single (μg)		†1·1–5·0 (2)	?3·8–5 (2)		††3·0 (1)
Histamine acid phosphate					
Single (μg)	*19·2–43 (3)	*8–11 (3)	*20–43 (3)	*4·3–10 (3)	—

change of EWL with changing T_{sk}. Because these two measurements were made on different samples of perfused skin, it may not be possible with each species to relate the two directly, or to extrapolate outside the ranges of T_{sk} in which they were measured. For example, in sheep the values listed indicate that a 6° C fall in T_{sk} would reduce EWL to zero; but there was no evidence that this would actually occur.

Sweating responses

Three types of evidence indicated that active sweating had occurred in response to certain of the pharmacological stimuli: (i) a change in EWL in excess of that which could be attributed to a passive effect of a change in T_{sk}; (ii) a positive starch–iodine reaction (Wada, 1950): the appearance of black spots in a starch–iodine mixture applied to the skin surface; and (iii) the appearance of numerous large droplets of sweat on the hairy surface of perfused horse skin.

The sixty-one perfused skin samples were from fifty-one animals. The perfusions of the skin from the three horses and the donkey were all entirely satisfactory. Of the remaining fifty-seven perfusions of ruminant skin, nineteen could be considered entirely satisfactory, twenty only moderately so and eighteen quite unsatisfactory. Adrenaline elicited a consistent sweating response in all species and was therefore used as a test injection. Only when a sweating response could be elicited by adrenaline were the sweat glands considered to be responsive, and all negative responses reported in Table 1 occurred when a subsequent injection of adrenaline was effective.

A summary of the sweating responses of the skin of the different species to drugs administered by arterial perfusion is given in Table 1. The drug doses cannot be related to a particular mass of perfused tissue because this could not be accurately determined. In experiments in which the area of perfused trunk skin was approximately determined by injection of Indian ink at the termination of the perfusion, the mass of tissue perfused was of the order of 100 g. When the EWL was measured with a capsule (Figs. 2, 5*a*, 6), the calculated values of EWL per unit area were often 10 times greater than when EWL was measured from a whole ear or tail (Figs. 3, 4, 5*b*). Terminal injections of Indian ink indicated that this difference was because only part of the tail or ear skin was being perfused. Since the changes in EWL following administration of drugs were consistent in the various tissues from each species and in the first and second series of experiments, conclusions have been drawn without reference to the tissue used.

Sheep. When a single dose of adrenaline was administered to sheep skin, the injection if effective induced a brief dose-dependent increase in the rate of EWL which began within 60 sec of the drug reaching the tissue. The

lowest effective dose was 0·1 μg per 100 g perfused tissue, but in many preparations adrenaline doses 10 or even 100 times larger were necessary to elicit a measurable sweating response. Repeated injections of adrenaline elicited progressively smaller discharges of sweat but if the perfusion was continued for an hour or more without drugs, a partial recovery of the response sometimes occurred (Fig. 2). A similar response to repeated injections of adrenaline was observed in conscious sheep by Johnson (1971). Noradrenaline was invariably less effective in stimulating sweat discharges than adrenaline (Fig. 2) but so long as a preparation remained sensitive to adrenaline, noradrenaline was always effective if given in sufficient doses.

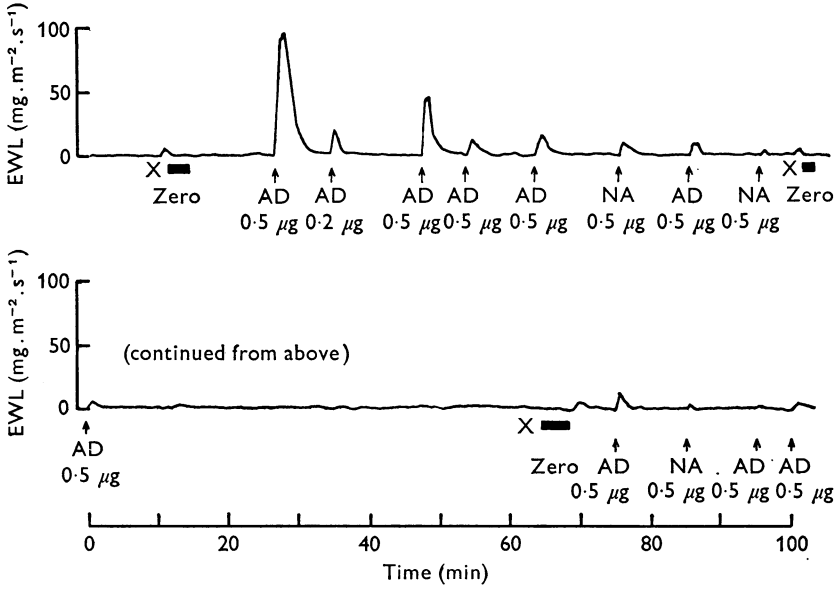

Fig. 2. EWL measured with a capsule on inguinal skin from a Clun Forest wether. The lower trace is a continuation of the upper. At the points marked X, artifacts appear in the trace during testing. In this and other Figures, the record above the solid bars corresponds to zero EWL, AD = adrenaline, NA = noradrenaline, ISOP = isoprenaline, CARB = carbachol, 5-HT = 5-hydroxytryptamine, OXY = oxytocin, BK = bradykinin, HIST = histamine, ATR = atropine and PEMP = pempidine.

When noradrenaline or adrenaline was added to the perfusate continuously for 10–20 min, a discharge of sweat occurred but the sweat output was not maintained. In some instances a slight elevation of EWL remained after the initial discharge, but when allowance was made for the increased EWL attributable to the associated rise in T_{sk}, the calculated output of sweat was small or more usually negligible (Fig. 3*a*, *b*).

Single injections of isoprenaline, carbachol, 5-HT, bradykinin, oxytocin and histamine, and continuous administrations of isoprenaline or carbachol were without measurable effect on EWL (Fig. 3).

Goats. Almost all the responses of the sweat glands of goat skin to drugs were similar to those observed in sheep. Injections of adrenaline or noradrenaline elicited abrupt and transient discharges of sweat, and prolonged infusions of adrenaline or noradrenaline usually did likewise (Fig. 4),

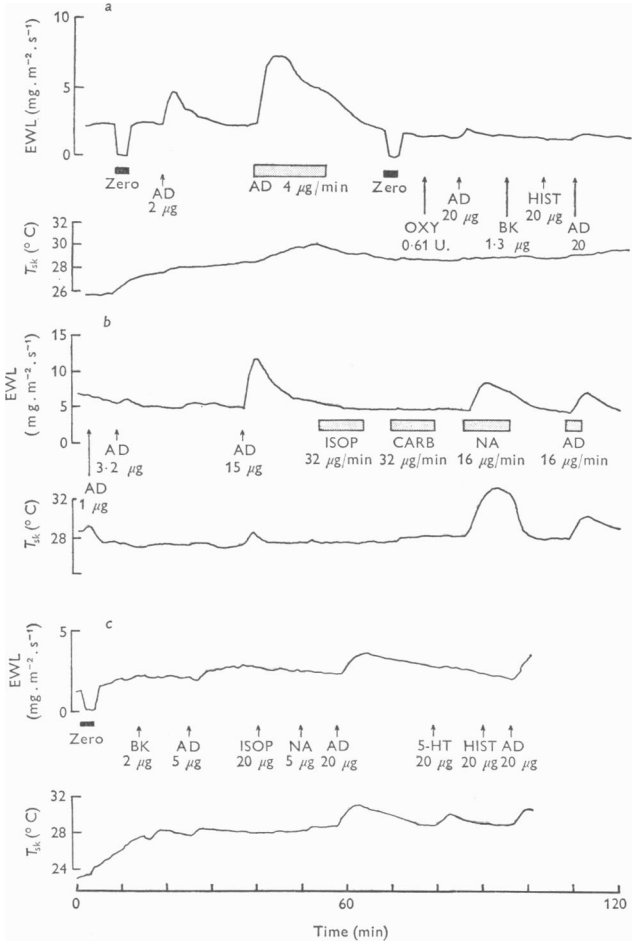

Fig. 3. EWL and T_{sk} of (*a*) the tail from a Welsh Mountain wether, (*b*) the ear from a Welsh Mountain wether and (*c*) the tail from a Dalesbred wether.

although in one experiment the sweat discharge during an infusion of noradrenaline was greater than could be attributed to the observed rise in T_{sk}. Isoprenaline, carbachol, 5-HT, oxytocin and histamine injections

were consistently ineffective. In two of four experiments in which bradykinin was administered, injections of 1·1 and 5 μg of the drug provoked a large discharge of sweat similar in onset and appearance to that induced by adrenaline; in another two experiments, administrations of 2·5 and 3·2 μg bradykinin were ineffective.

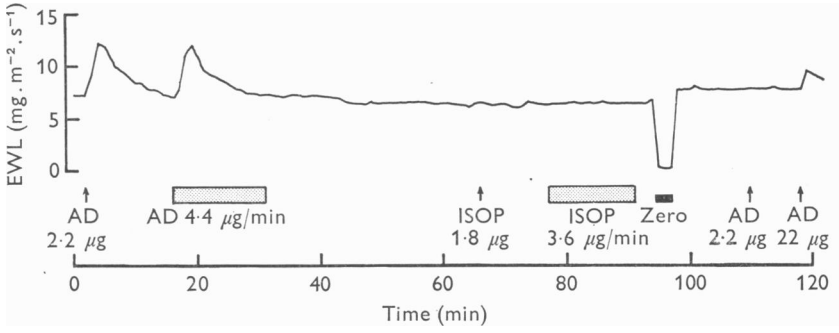

Fig. 4. EWL of the tail from a Welsh × Saanen goat.

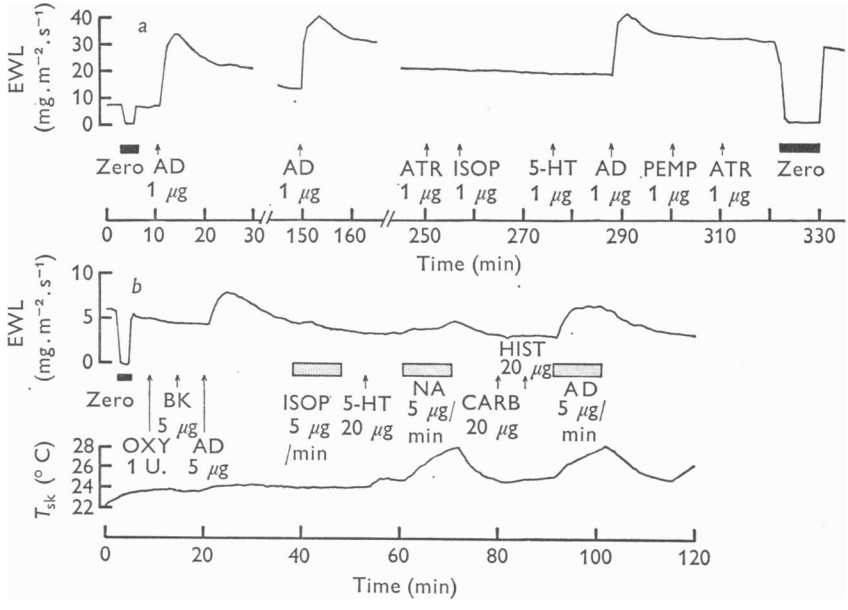

Fig. 5. *a*, EWL measured with a capsule on flank skin from a Jersey cow; *b*, EWL and T_{sk} of the ear from a Jersey calf.

Cattle. Single doses of adrenaline added to the perfusate of bovine skin elicited sweat discharges as rapid in onset as those observed in sheep and goat skin but lasting considerably longer (Fig. 5*a*). A similar protracted

sweat discharge following adrenaline injection into a conscious cow is reported by Allen & Bligh (1969). The sensitivity of cattle skin to adrenaline was of the same order as that recorded in sheep and goat skin, as was the relatively lower sensitivity to noradrenaline. When adrenaline was infused continuously for 10–20 min, the sweat output from cattle skin was maintained (Fig. 5*b*); when noradrenaline was infused continuously, the effect was small and could not with certainty be shown to exceed the passive rate of EWL (Fig. 5*b*).

Injections or infusions of isoprenaline and carbachol were without measurable effect on sweat output, as were injections of 5-HT, oxytocin and histamine (Fig. 5). Very small discharges of sweat followed two of the four administrations of bradykinin (Table 1).

Horses and donkey. The horse skin remained very sensitive to adrenaline and to isoprenaline and relatively insensitive to noradrenaline during perfusions lasting many hours. Fig. 6 shows the effects on two skin samples of injections or infusions of various drugs. Noradrenaline induced rapid but small discharges of sweat; adrenaline induced large and sometimes clearly biphasic sweat discharges; isoprenaline induced large sweat discharges which began only after 2–3 min administration of the drug but were then maintained while the drug was infused. Coincident with the rise in EWL was the appearance of numerous droplets of sweat on the perfused skin near, and presumably under, the sweat collection capsule. The responses to noradrenaline, oxytocin and bradykinin and the initial part of the biphasic response to adrenaline were consistently more rapid in onset than the response to isoprenaline or than the second phase of the response to adrenaline. Carbachol infusions at a very high dose rate given in one experiment evoked a small continuous discharge of sweat, but usually no response was measurable. Injections of histamine and 5-HT were likewise ineffective. Injections of large single doses of oxytocin and bradykinin (Fig. 6, Table 1) led consistently to the discharge of small amounts of sweat.

Vasomotor responses

In all species, adrenaline and noradrenaline administered in low doses induced vasoconstriction without sweating; at higher doses, a vasoconstriction preceded the discharge of sweat. The vasoconstrictive actions of noradrenaline were greater than those of adrenaline although the sudorific actions were less. 5-HT caused a vasoconstriction usually as intense as that caused by an equal dose of adrenaline or noradrenaline, and oxytocin caused a transient vasoconstriction. The vasomotor effects of carbachol, isoprenaline, histamine and bradykinin were weak and variable although the effect was most commonly a slight vasodilatation.

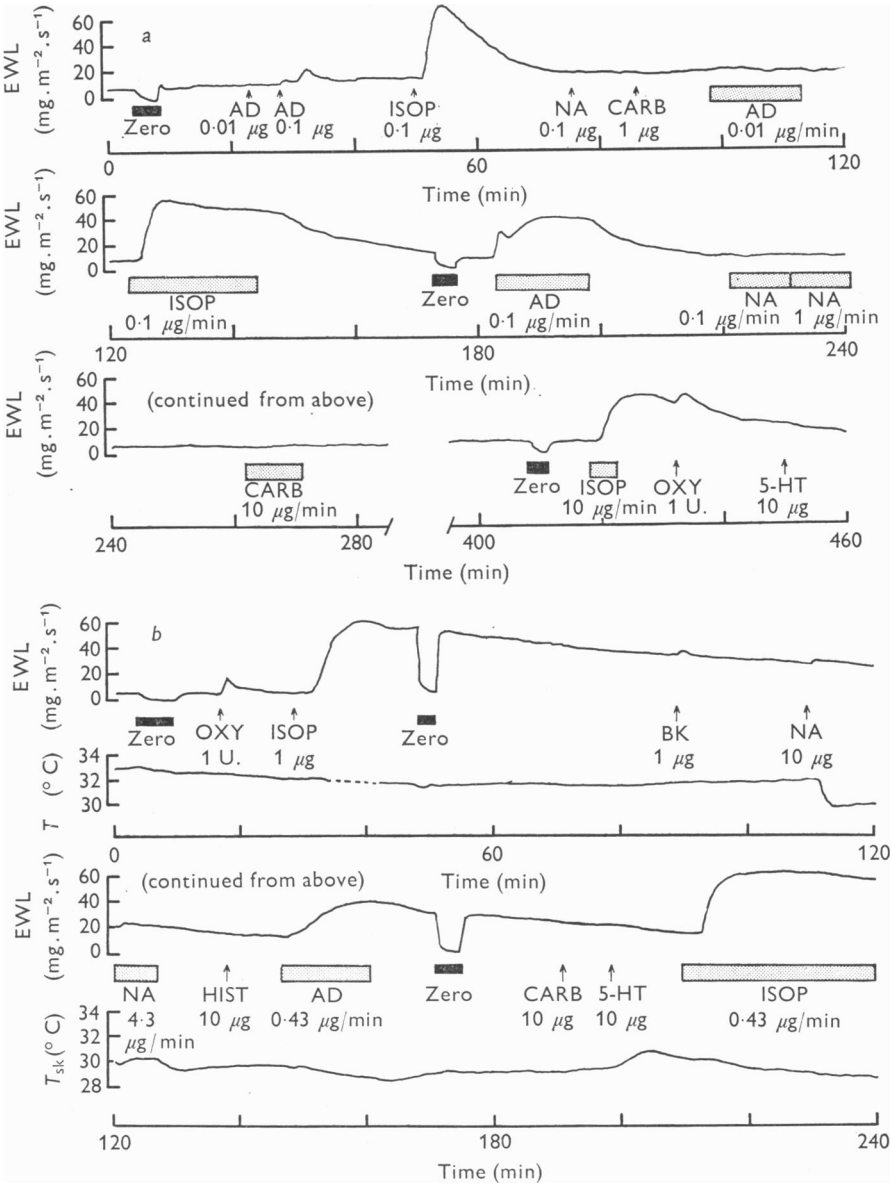

Fig. 6. *a*, EWL measured with a capsule on flank skin from a horse. The traces run consecutively from top to bottom. *b*, T_{sk} and EWL, measured with a capsule, on flank skin from a horse. The lower traces are continuations of the upper ones.

DISCUSSION

A study of sweating in isolated perfused skin is of particular value if the tissue responds as it would in an intact conscious animal. Indications that this was more or less so in the present preparations were: (i) the basal rates of EWL were of the order reported previously in sheep (Brook & Short, 1960), goats (Allen & Bligh, 1969) and cattle (Alvarez, Hahn & Johnson, 1970); (ii) EWL increased in response to administration of drugs which in similar doses cause sweating in conscious animals (Jenkinson, 1973); (iii) the patterns of sweating following drug administration in isolated skin were similar in certain respects to those reported in conscious animals (Allen & Bligh, 1969; Jenkinson, 1973); (iv) these increases in EWL coincided with the appearance of droplets of sweat on the skin of horses or of the demonstrable presence (Wada, 1950) of an aqueous solution at the base of the wool or hair fibres in the other species; and (v) secretory cells apparently remained active during the perfusion, since sweat discharge and presumably sweat secretion could still be elicited in some experiments on horse and ox skin after many hours of perfusion, and in other experiments on sheep a sweating response which had disappeared after repeated testing later reappeared after a period of infusion without stimulation (Fig. 2).

The samples of horse skin studied, and to a lesser extent that of donkey skin, were highly vascularized; all the samples examined were consistently responsive and highly sensitive to drugs, and remained responsive for many hours of perfusion. In contrast, only about one third of the perfusions of sheep, goat and cattle skin were entirely successful, one third were moderately successful and one third were entirely unsuccessful. Variations in the delivery of perfusate to the sweat glands was a likely, but probably not the only, cause of this variability. At the completion of many experiments, Indian ink was injected into the tissue via the arterial cannula; poor penetration of ink to the sweat collection areas was commonly observed in those tissues from which sweating responses had been indifferent or feeble. However, in other unresponsive tissues, ink penetration was quite satisfactory, so the sweat glands themselves are presumed to have been adequately perfused but insensitive. Both Robertshaw (1968) and Allen & Bligh (1969) have reported big differences between animals in the sensitivity of their sweat glands to heat and I.V. drug administration, and the tissue insensitivity observed may have been a further manifestation of this.

Vasomotor and sudomotor responses were sometimes confounded because vasoconstrictive drugs increased both T_{sk} and EWL. However, there are several reasons why the increases in EWL designated here as

sweating are not attributable simply to changes in T_{sk}. Firstly, considerable rises in EWL were shown to have occurred even after corrections had been made for the changes in T_{sk}. Secondly, in the skin of all animals, 5-HT was a powerful vasoconstrictive agent with a greater vasoconstrictive effect than adrenaline, yet 5-HT elicited no more than a small fraction of the increase in EWL caused by sudorific drugs (Figs. 3*c*, 5, 6*b*). Thirdly, in the horse and donkey skin, isoprenaline induced copious sweating in the absence of any rise in T_{sk} (Fig. 6*b*).

Table 1 records which drugs were effective in causing sweating in isolated perfused skin; the findings are in general agreement with results previously observed in conscious animals and reviewed by Jenkinson (1973). Although the effective doses of drugs could not be related quantitatively to a tissue weight because the mass of perfused tissue could not be accurately measured, the trends were nevertheless clear that ruminant sweat glands were stimulated most effectively by the α-adrenergic agonists, adrenaline and noradrenaline, and the equine sweat glands by the β-adrenergic agonists, adrenaline and isoprenaline. Sweat glands sometimes discharged briefly in response to injections to bradykinin and in a few instances to very large doses of oxytocin. No sweating was ever induced by histamine or 5-HT, and carbachol was also ineffective except in horses in which very large doses elicited a small output.

Bligh (1967) and Alvarez *et al.* (1970) have offered hypotheses to explain the patterns of sweating induced by the administration of sudorific drugs, and these can be tested with the results reported. Alvarez *et al.* (1970) suggested that, in cattle, sweat expulsion was a passive consequence of haemodynamic changes which induced movement of capillary fluid into the interstitial space so influencing the sweat glands. The authors proposed that during heat exposure or the systemic administration of adrenaline which increases heat production, this extravasation of fluid results from cutaneous vasodilatation; following local injections of vasoconstrictive drugs, they propose that extravasation of capillary fluid results from vasoconstriction. The possibility that sweat discharges in the present experiments were secondary to haemodynamic changes following vasomotor responses is remote; for in all species 5-HT induced a strong vasoconstriction but no sweat discharge, and conversely, isoprenaline induced a sweat discharge in the horses and the donkey with negligible alteration in vasomotor tone. Furthermore, when histamine was injected or the perfusion pressure was increased to twice normal, some extravasation of intravascular fluid would be expected, but following neither of these treatments was there any increase in sweat output. Since neither vasoconstrictor nor vasodilator responses, nor a presumed movement of fluid into the interstitial spaces, was correlated with the discharge of

sweat, the present results lend no support to the hypothesis of Alvarez *et al.* (1970) as an explanation of sweat discharge in cattle or any other of the species studied.

Bligh (1967) suggested that the patterns of sweat discharge in different species might be determined by a combination of two mechanisms, a continuous but variable rate of secretion of fluid into the sweat gland lumen and an intermittent expulsion of sweat on to the skin surface brought about by contraction of the myoepithelium. A feature of this hypothesis is that it is adequate to explain any discharge pattern, because any continuous output of fluid can be attributed to overflow of secretion and any fluctuation in sweat output to myoepithelial activity, but the ability to provide such explanations does not constitute verification of the hypothesis. Two methods which could be used to test the hypothesis are (i) the microscopic observation of separate contributions of secretion and myoepithelial contraction to sweat output, and (ii) pharmacological dissociation of secretory and myoepithelial components of the sweating process by the activation or inactivation of one of them. The first of these possibilities has been investigated with isolated perfused skin; sweat gland contractions induced by sudorific drugs are believed to have been seen, but photographic recording of these events has not so far been possible (unpublished observations). The alternative procedure for verification of the hypothesis by independent activation of two separate components of the sweating system has been examined in the present experiment and the results lend some support to Bligh's proposals.

The sweat glands of sheep and goats always discharged as if by a single expulsion of fluid; even when the most effective sudorific agent, adrenaline, or any other of the possible transmitters studied was infused continuously, the sustained rate of sweating was little if at all greater than the background EWL. Possible explanations of these brief discharges of sweat are that they are due to the rapid activation of an α-adrenergic secretory process which quickly fatigues, or perhaps more likely, to myoepithelial contraction. In the horse and donkey skin a clearer pattern of responses was apparent. Bradykinin, oxytocin, or the α-adrenergic agonist, noradrenaline, evoked only brief discharges of sweat even, in the case of noradrenaline, when infused continuously (Fig. 6*b*). Infusions of the β-adrenergic agonist, isoprenaline, on the other hand, resulted in a continuous output of fluid (Fig. 6*a*, *b*). Infusions of adrenaline, a mixed α- and β-adrenergic agonist, evoked in several instances (Fig. 6*a*, *b*) a biphasic response consisting of an early rapid discharge followed by a large continuous outflow. When sweat production was elevated by the action of isoprenaline, oxytocin could evoke an extra increase in output (Fig. 6*a*) as if by a superimposed myoepithelial contraction. In these preparations of horse and donkey skin,

a β-adrenergic system appeared to control sweat production, and an α-adrenergic system, activated also by bradykinin and oxytocin, to control a mechanism capable of single rapid discharges, presumably the myoepithelium. This explanation could also be applied to the observations on sweating in sheep and goats, if the β-adrenergic secretory system in these species is assumed weak or absent.

The pattern of sweating observed in cattle was not explicable in these terms. Adrenaline and, to a lesser extent, noradrenaline, could induce a continuous discharge of sweat (Fig. 5*b*) and this was apparently not due to β-receptor activation since isoprenaline administrations were ineffective. Unlike the situation in the horse, donkey, sheep and goat, the secretory mechanism in cattle was apparently activated by α-adrenergic stimulation. Since an α-adrenergic mechanism evidently also controlled vasomotor responses, some interaction between the sudomotor and vasomotor control may occur, either by a vasoconstriction limiting local perfusion or by the activation of both effector mechanisms by a common transmitter. This might explain why the sweating responses to single injections of adrenaline took considerably longer to subside in cattle than in other species (Fig. 5*a*). Myoepithelial contraction of the glands may have occurred at the beginning of the sweating response in cattle, but this was uncertain. Robertshaw (1971) has described α- and β-adrenergic, and also cholinergic, modes of control of sweat glands in different species. It appears possible that there are, in addition, sweat gland effector systems, such as those in equidae, in which both α- and β-adrenergic mechanisms play a role.

The present experiments have shown that perfused skin from a number of species will sweat in response to the administration of sudorific drugs, and that some features of the specific patterns of sweating evident in conscious animals remain after isolation of the skin from the animal. Furthermore, by making use of specific drug actions, it has appeared possible to dissociate two mechanisms contributing to sweat discharge. With more refined techniques of investigation of isolated perfused skin, such as the use of micropuncture techniques for the injection of marker substances into the sweat duct, collection of primary secretions from the gland lumen, and measurement of secretory potentials, the physiological basis of the species differences in sweat gland function may be revealed.

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